(54) Title: REGULATOR REGIONS OF G PROTEINS

(57) Abstract

A molecule having a peptide sequence of 80 or fewer amino acid residues containing the amino acid sequence of an anticouplone of any of the G proteins, which molecule is useful for inhibiting activation of the G protein by its G-coupled receptor.

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REGULATOR REGIONS OF G PROTEINS

The field of the invention is cell receptor-based
signal transduction.

5   Background of the Invention

Guanine nucleotide-binding proteins (G proteins)
are a family of signal transducers which are found
associated with the plasma membrane within eukaryotic
cells, and which act to relay signals from certain cell-
10 surface receptor molecules (G-coupled receptors) to
effectors in the plasma membrane. G proteins are
functional dimers consisting of α (Gα) and $\beta\gamma$ (G$\beta\gamma$)
subunits (Stryer et al., Ann. Rev. Cell Biol. 2:391-419,
1986). Some presently-known members of this family
15 include the G proteins termed G_{o1}, G_{o2}, G_{i1}, G_{i2}, G_{i3}, G_s,
G_q, and the G_q-related family which presently includes
G_{11}, G_{12}, G_{13}, G_{14}, G_{15}, and G_{16} (see, e.g., Kaziro,
"Structures of the Genes Coding for the α Subunits of G
Proteins", Chapter 11 in ADP-ribosylating Toxins and
20 G Proteins (Moss, J., and Vaughan, M. eds.) pp189-206,
American Society for Microbiology, Washington, D.C.,
1988). Each individual G protein is characterized as
such by the particular α subunit species (e.g., $G_{o1}\alpha$,
$G_{o2}\alpha$, etc.) which forms the α portion of the dimeric
25 protein. In their unstimulated condition, G proteins are
$\alpha\beta\gamma$ forms bound to guanosine diphosphate (GDP); upon
receptor stimulation they dissociate into GTP-bound Gα
and nucleotide-free G$\beta\gamma$, leading to the activation of the
next effector protein in the cascade. In at least one
30 case, this activation results in the opening of a calcium
channel in the membrane, thereby increasing calcium
influx in the cell (Kojima et al., Biochem. Biophys. Res.
Commun. 154:9-19, 1988; Matsunaga et al., Am. J. Physiol.
255:C442-C446, 1988).

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Okamoto et al. (Cell 62:709-717, 1990; hereby
incorporated by reference) identified the region of one
G-coupled receptor protein (insulin-like growth factor II
receptor, or IGF-IIR) which directly interacts with and
5   activates G_{i2}; this region of IGF-IIR is sometimes
referred to as that receptor's "couplone". A synthetic
peptide (termed "Peptide 14") having a sequence identical
to that of IGF-IIR's couplone was found to bind to and
activate G_{i2}. Studies on synthetic variants of this
10   peptide permitted Okamoto et al. to posit a critical
peptide length (10 to 17 residues) and a motif for the
couplone: two B's at the amino terminus of the peptide,
and $B-B-X-B$ or $B-B-X-X-B$ at the carboxy terminus, where B
is a basic amino acid (Lys, Arg, or His) and X is any
15   nonbasic amino acid. In another G-coupled receptor, β_2-
adrenergic receptor (β2AR), a G_s-activator region termed
βIII-2 has been identified in the third intracellular
loop of β2AR (Okamoto et al., 1991a).

Summary of the Invention

20   Applicants have identified the precise region on
each of the known eukaryotic G proteins which interacts
directly with the associated G-coupled receptor proteins.
This region, termed the "anticouplone" or regulator
region of the G protein, has been found to be critical to
25   the function of the G protein, and therefore to signal
transduction by the associated receptor. The invention
thus encompasses a molecule of 80 or fewer amino acid
residues (preferably 50 or fewer, more preferably 40 or
fewer, and most preferably 30 or fewer) containing the
30   amino acid sequence of an anticouplone of any of the G
proteins (e.g., a mammalian G protein such as G_{o1}, G_{o2},
G_{i1}, G_{i2}, G_{i3}, G_q, and G_s). The anticouplone region of a
given G protein is located near the carboxy terminus of
the G protein, from the 4th residue from the carboxy

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terminus at least to the 18th residue from that terminus,
inclusive, and preferably to approximately the 27th
residue from that terminus, inclusive. The molecule may
contain an amino acid sequence defined by

5   DXXXXVIIKNNLXXC (SEQ ID NO: 1),
where X denotes any amino acid (up to two of which may be
deleted) and the other letters represent specific amino
acids according to the standard single-letter amino acid
code. More preferably, the peptide sequence is defined

10 by
               DAVTXVIIKNNLKXC (SEQ ID NO: 2).

Specific examples of such a sequence include
               DAVTDVIIKNNLKDC (SEQ ID NO: 3),
             DTKNVQFVFDAVTDVIIKNNLKDC (SEQ ID NO: 4),

15   DAVTDVIIKNNLKEC (SEQ ID NO: 5), and
             DTKNVQFVFDAVTDVIIKNNLKEC (SEQ ID NO: 6).
Another version of the molecule of the invention includes
the sequence
               DAVTXIIIAXNLRXC (SEQ ID NO: 7);

20 examples include
               DAVTDIIIANNLRGC (SEQ ID NO: 8),
             NNIQVVFDAVTDIIIANNLRGC (SEQ ID NO: 9),
              DAVTDIIIAKNLRGC (SEQ ID NO: 10), or
             NNIQFVFDAVTDIIIAKNLRGC (SEQ ID NO: 11).

25 Other molecules of the invention include a sequence
               NDCRDIIQRMHLRQY (SEQ ID NO: 12),
             DTENIRRVFNDCRDIIQRMHLRQY (SEQ ID NO: 13),
               AAVKDTILQLNLKEY (SEQ ID NO: 14), or
             DTENIRFVFAAVKDTILQLNLKEY (SEQ ID NO: 15).

30   The molecules of the invention may be employed in
a method of inhibiting activation of a given G protein by
a G-coupled receptor on a cell, which method includes the
step of contacting the cell with, or otherwise
introducing into the cell, a molecule containing the

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anticouplone sequence of the G protein, which molecule
contains 40 or fewer amino acid residues. This method
may be carried out, for example, by local or systemic
administration of the appropriate peptide or modified

5 peptide, or by introducing into the cell a nucleic acid
encoding the anticouplone peptide together with all
promoters and other elements necessary for expression of
the peptide from the nucleic acid. Techniques for
constructing such a nucleic acid and introducing it into

10 the target cells are well known to those of ordinary
skill in the art. Where the G protein of interest is G_{i1},
G_{i2}, or G_{i3}, the targeted receptor may be, for example,
insulin-like growth factor II receptor (IGF-IIR),
muscarinic acetylcholine receptor (mAChR), somatostatin

15 receptor, D_2-dopamine receptor, α_2-adrenergic receptor,
adenosine receptor, thrombin receptor, TGFβ receptor, or
any other receptor which is functionally coupled to one
of those G proteins. Where the G protein of interest is
G_{o1} or G_{o2}, the receptor may be, for example, γ-butyric

20 acid (GABA) receptor, somatostatin receptor, muscarinic
acetylcholine receptor (mAChR), α_2-adrenergic receptor,
adenosine receptor, thrombin receptor, or TGFβ receptor.
Examples of G_s-linked receptors include β_2-adrenergic
receptor, glucagon receptor, IL-1 receptor, and D_1-

25 dopamine receptor; while examples of G_q-linked receptors
include the PTH/PTHrP receptor, calcitonin receptor,
endothelin receptor, T cell receptor, angiotensin
receptor, platelet activating factor receptor, and
thromboxane A_2 receptor.

30   Other features and advantages of the invention
will be apparent from the detailed description set forth
below, and from the claims.

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Brief Description of the Drawings

Figs. 1a-1d is a set of graphs illustrating the effect of ACG_{i2} on G protein activation. (A) Effects of ACG_{i2} and control peptides on peptide 14-induced G_{i2}
5 activation. (B) Effect of ACG_{i2} on TrM-peptide 14-induced G_{i2} activation and βIII-2-induced G_s activation. (C) Effect of ACG_{i2} on G_{i1} and G_{i3} activation. (D) Effect of ACG_{i2} on MIII-induced G_{i2} and G_o activation.

Figs. 2a-2b is a pair of graphs illustrating the
10 mode of action of ACG_{i2}. (A) Dose/response relationship of peptide 14 action in the presence of various concentrations of ACG_{i2}. (B) Effect of ACG_{i2} on activation of 1 nM G_{i2} induced by peptide 14.

Figs. 3a-3c is a set of graphs illustrating the
15 structure-function relationship of ACG_{i2} on peptide 14-induced G_{i2} activation. (A) Effect of ACG_{i2} and high potency group variants. (B) Effect of low potency group variants. (C) Effect of intermediate potency group variants.

20   Fig. 4 is a bar graph illustrating the effect of ACG_{i2} on mAChR-G_{i2} coupling.

Fig. 5 is a schematic drawing illustrating a proposed mechanism whereby the activation state of G_{i2} is affected by peptide 14 and/or ACG_{i2} peptide.

25   Fig. 6 is a set of three photomicrographs illustrating the inhibitory effect of ACG_{i2} on serum-induced CHO cell growth.

Fig. 7 is an autoradiograph of an SDS-PAGE analysis illustrating the effect of ACG_{o2} on TGFβ-induced
30 fibronectin secretion from kidney cells.

Fig. 8 is a pair of photomicrographs illustrating the effect of ACG_{i2} on cardiac fibrosis in myopathic hamsters.

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Detailed Description

The anticouplones of the invention were discovered by analyzing the sequences of the known G proteins to identify the region on each which was deemed likely to
5 function as an anticouplone, and then testing each candidate anticouplone, and variants thereof, in appropriate *in vitro* and *in vivo* assays.

Identification of Candidate Anticouplone Sequences

On the assumption that, in order to account for
10 the specificity of each receptor for a given type of G protein, the $G\alpha$ subunit of a given G protein must participate in the direct interaction with the associated receptor's couplone, it was postulated that the anticouplone, or signal-acceptor domain, of the G protein
15 dimer would be located, at least in part, on the $G\alpha$ subunit. Certain criteria, described below, were then established to define the putative signal-acceptor domain on each G protein's α subunit. These criteria were initially applied to determine the anticouplone of $G_{i2}\alpha$,
20 as follows: Although a recent publication suggested otherwise (Okuma et al., J. Biol. Chem. 267:14826-14831, 1992), a number of lines of evidence indicated to Applicant that at least a portion of the anticouplone sequence would be located at or near the carboxy-terminal
25 end of the G protein (Okamoto et al., Proc. Natl. Acad. Sci. USA 88:3020-8023, 1991; Ui et al., Adv. Cyclic Nucleotide Protein Phosphorylation Res., 17:145-151, 1984; Rall et al., FEBS Lett. 224:365-371, 1987; Masters et al., Science 241:448-451, 1988; Moss and Vaughan,
30 eds., American Society for Microbiology, 1988, pp.207-224; Simonds et al., Proc. Natl. Acad. Sci. USA, 86:7809-7813, 1989; McClue et al., FEBS Lett. 269:430-434, 1990; Shenker et al., J. Biol. Chem. 266:9309-9313, 1991; Murray-Whelan et al., J. Biol. Chem. 267:2960-2965, 1992;
35 Meinkoth et al., J. Biol. Chem. 267:13239-13245, 1992;

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Okamoto and Nishimoto, Proc. Natl. Acad. Sci. USA
88:8020-8023, 1991; and Sullivan et al., Nature 330:758-
760, 1987). Therefore, the first criterion specified
that the candidate signal-acceptor region be located in
5 the carboxy-terminal portion of $G_{i2}\alpha$.

Within the carboxy-terminal portion of $G_{i2}\alpha$,
Applicant focused on the region Asp338-Asp351 (SEQ ID NO:
16). This region has a primary structure in which the
negatively charged residues are oriented as matching the
10 positively charged basic residues essential for the G_{i2}-
activating function of Peptide 14. The structural
determinants for this function of Peptide 14 were
hypothesized to be (i) two basic residues at its amino
terminus and (ii) a carboxy terminal motif of *B-B-X-B* or
15 *B-B-X-X-B*. Asp338-Asp351 (DAVTDVIIKNNLKD) (SEQ ID NO:
16) is the only region of G_{i2} that meets all of these
criteria. This sequence was therefore tentatively
identified as the $G_{i2}\alpha$ anticouplone. It was found that
$G_{i1}\alpha$ contains the same 14 residues at a corresponding
20 region, while $G_{i3}\alpha$ contains the almost identical sequence
DAVTDVIIKNNLKE (SEQ ID NO: 17), in which the N-terminal
Asp is replaced by Glu. When the four criteria are
applied to $G_{o1}\alpha$ and $G_{o2}\alpha$, the resulting anticouplone
sequences are defined by DAVTDIIIANNLRGC (SEQ ID NO: 8)
25 and DAVTDIIIAKNLRGC (SEQ ID NO: 10), respectively. The
anticouplone sequence of G_s is NDCRDIIQRMHLRQY (SEQ ID
NO: 12), while that of G_q is AAVKDTILQLNLKEY (SEQ ID NO:
14).

The above reasoning was used to identify a
30 putative receptor-binding domain on each individual $G\alpha$
species. Later experiments carried out with synthetic
peptides representing these putative receptor-binding
domains have suggested that, rather than receptor-binding
domains, these particular regions of the $G\alpha$ polypeptides
35 may serve as $G\alpha$-activating regulator regions. This

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completely unexpected finding is discussed in detail below.

Synthesis of Anticouplone Peptides

        The peptides used in this study were synthesized
5 and purified as described (Okamoto et al., 1990; Okamoto
and Nishimoto, 1991; Okamoto et al., 1991a; Okamoto and
Nishimoto, 1992). The following peptides were prepared:

        1. ACG_{i2}, corresponding both to $G_{i1}\alpha$ Asp337-
Cys351 and to $G_{i2}\alpha$ Asp338-Cys352 (DAVTDVIIKNNLKDC; SEQ ID
10 NO: 3). The fact that pertussis toxin, which catalyzes
ADP-ribosylation of Cys352, attenuates G_{i2} activation
produced by peptide 14 (Okamoto et al., 1990, *supra*) as
well as by IGF-IIR (Nishimoto et al., J. Biol. Chem.
264:14029-14038, 1989), prompted the inclusion of Cys352
15 in this peptide.

        2. ACG_{i3} (DAVTDVIIKNNLKEC; SEQ ID NO: 5).

        3. ACG_{o1} (DAVTDIIIANNLRGC; SEQ ID NO: 8).

        4. ACG_{o2} (DAVTDIIIAKNLRGC; SEQ ID NO: 10).

        5. ACG_{s} (NDCRDIIQRMHLRQY; SEQ ID NO: 12).

20   6. ACG_{q} (AAVKDTILQLNLKEY; SEQ ID NO: 14).

        7.   Alternative G_{o1} peptide
NNIQVVFDAVTDIIIANNLRGC (SEQ ID NO: 9).

        8.   Alternative G_{o2} peptide NNIQFVFDAVTDIIIAKNLRGC
(SEQ ID NO: 11).

25   9.   Alternative G_{s} peptide
DTENIRRVFNDCRDIIQRMHLRQY (SEQ ID NO: 13).

        10.   Alternative G_{q} peptide
DTENIRFVFAAVKDTILQLNLKEY (SEQ ID NO: 15).

        11.   Control peptide Glu297-Asp310
30 (EAASYIQSKFEKLN; SEQ ID NO: 18) corresponding to $G_{i2}\alpha$
Glu297-Asp310.

        12.   Control peptide Lys346-Phe355 (KNNLKDCGLF;
SEQ ID NO: 19), representing the carboxy-terminal 10
residues of $G_{i2}\alpha$.

35   13. A_{1} variant (AAVTDVIIKNNLKDC; SEQ ID NO: 20).

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14. A$_2$ variant (DAVTAVIIKNNLKDC; SEQ ID NO: 21).

15. A$_3$ variant (DAVTDVIIKANLKDC; SEQ ID NO: 22).

16. A$_4$ variant (DAVTDVIIKNALKDC; SEQ ID NO: 23).

17. A$_5$ variant (DAVTDVIIKNNLKAC; SEQ ID NO: 24).

18. p9 variant (DAVTDVIIK; SEQ ID NO: 25).

19. p11 variant (DVIIKNNLKDC; SEQ ID NO: 26).

20. del C variant (DAVTDVIIKNNLKD; SEQ ID NO: 16).

21. Peptide 14 (RVGLVRGEKARKGK; SEQ ID NO: 27), the 14-residue couplone portion of the G$_{i2}$-coupled receptor protein IGF-IIR.

22. TrM peptide 14 (LTACLLTLLLYRVGLVRGEKARKGK; SEQ ID NO: 28), peptide 14 linked to the native transmembrane domain of IGF-IIR.

23. βIII-2 (RRSSKFCLKEHKALK; SEQ ID NO: 29), the 15-residue couplone region of the G$_s$-coupled receptor β2AR.

24. MIII peptide (RNQVRKKRQMAARERKVTR; SEQ ID NO: 30), the 19-residue couplone region of the G$_i$/G$_o$-coupled M$_4$ muscarinic acetylcholine receptor (M$_4$AChR).

Designed but not prepared were two additional peptides in the series:

25. Alternative G$_{i1}$/G$_{i2}$ peptide DTKNVQFVFDAVTDVIIKNNLKDC (SEQ ID NO: 4), and

26. Alternative G$_{i3}$ peptide DTKNVQFVFDAVTDVIIKNNLKEC (SEQ ID NO: 6).

In Vitro, Cell-free Assays of Anticouplone Peptides

Materials and Methods

The in vitro assays described below utilize the couplone portion of each of a number of G-coupled receptors, instead of an intact receptor molecule, as a means for activating the corresponding anticouplone or intact G-protein. Except as noted below, G$_{i2}$ used in this

study was trimeric G_{i2} (i.e., $G_{i2}\alpha\beta\gamma$) purified from bovine
brain to near homogeneity (Katada et al., *FEBS Lett.*
213:353-358 (1987), stored in buffer A (20 mM hepes/NaOH
(pH 7.4), 1 mM EDTA, and 0.7% CHAPS), and diluted $\geq$ 10
5   fold for assays. The G_{i2} used for the reconstitution
experiment was trimeric G_{i2} purified from bovine spleen to
homogeneity and dissolved in buffer B (Morishita et al.,
1989). $G_{i3}\alpha$ (Morishita et al., *Biochem. Biophys. Res.
Commun.* 172:249-255 (1990), was purified from bovine
10 spleen and used in combination with 1.5-fold concentrated
$G\beta\gamma$ (Katada et al., 1987). This preparation of $G_{i3}\alpha$ was
10-20% contaminated by $G_{i1}\alpha$, but not by other G proteins.
Trimeric G_s and $G\beta\gamma$ purified from bovine brain to near
homogeneity (Katada et al., 1987) were also stored in
15 buffer A.

        mAChR (muscarinic acetylcholine receptor) was
purified from porcine brain to near homogeneity (Haga et
al., 1986) and dissolved in 0.5 M potassium phosphate
buffer (pH 7.0) containing 0.1% digitonin. This receptor
20 preparation was reconstituted with G_{i2} in phospholipid
vesicles using a gel filtration method as described
previously (Nishimoto et al., 1989). The concentrations
of mAChR and G_{i2} in the vesicles were 6.9 nM and 29 nM,
respectively.

25   *GTPγS binding Assay* - GTPγS binding to G proteins
was assayed at 37°C in 20 mM Hepes/NaOH buffer (pH 7.4)
containing 130 μM $MgCl_2$, 110 μM EDTA and 60 nM $[^{35}S]$GTPγS,
as described previously (Okamoto et al., 1990). GTPγS
binding to polypeptides was negligible. The total amount
30 of G protein was measured as maximal GTPγS binding at
room temperature, as described (Okamoto et al., Cell
67:723-730, 1991a). $[^{35}S]$GTPγS was purchased from DuPont-
New England Nuclear. Binding of GTPγS to G proteins
obeyed first-order kinetics according to the equation
35 $\ln[(BT - B)/B_T] = -k_{app}t$, where B is the binding at time t

and B_T is the total binding observable at an infinite
time. Thus, the apparent first-order rate constant for
GTPγS binding (k_{app}), which is equal to the slope of the
tangent to the GTPγS binding curve at time 0 and
5   represents the actual GTPγS binding rate, was calculated
from this equation.

Results

In the experiment illustrated in Fig. 1A, 10nM G_{i2}
was incubated with 30 μM peptide 14 (RVGLVRGEKARKGK; SEQ
10   ID NO: 27), the couplone portion of the IGF-II receptor,
in the presence of various concentrations of (□) ACG_{i2}
(DAVTDVIIKNNLKDC; SEQ ID NO: 3), (◊) the control $G_{i2}\alpha$
Glu297-Asp310 peptide (EAASYIQSKFEKLN; SEQ ID NO: 18), or
(◇) the control $G_{i2}\alpha$ Lys346-Phe355 peptide (KNNLKDCGLF;
15   SEQ ID NO: 19) for 5 min under the GTPγS binding assay
conditions described above, and GTPγS binding to G
proteins was measured. The effect of ACG_{i2} on G_{i2} in the
absence of peptide 14 is indicated by □. Percent
stimulation of GTPγS binding was calculated by
20   subtracting the basal fractional binding and dividing by
the stimulated fractional binding observed in the absence
of ACG_{i2}. The basal fractional binding (= 0%) was 0.39
mol GTPγS binding/mol G_i/5 min (5 min^{-1}). The 100%
increase represents the GTPγS binding of 0.85 (5 min^{-1}).
25   Values in all figures represent the mean ± S.E. of three
experiments.

Fig. 1A shows that ACG_{i2} peptide at 30μM inhibits
binding of GTPγS to G_{i2} in the presence of peptide 14 to
the basal level of binding exhibited by G_{i2} in the absence
30   of peptide 14. The IC_{50} was ≈3μM. ACG_{i2} had no effect on
GTPγS binding to G_{i2} in the absence of peptide 14. $G_{i2}\alpha$
has another region that satisfies (I)-(III): residues
Glu297-Asn310 (EAASYIQSKFEKLN; SEQ ID NO: 18). This
peptide, however, showed no effect on peptide 14-induced

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G_{i2} activation. As another control, a peptide
representing the carboxy-terminal 10 residues of $G_{i2}\alpha$
(KNNLKDCGLF; SEQ ID NO: 19) was synthesized and tested in
this assay. Unlike ACG_{i2}, this decapeptide exhibited no
5 inhibitory effect on peptide 14-induced G_{i2} activation.
These findings suggest that the antagonizing effect of
ACG_{i2} on G_{i2} activation is attributable to the specific
amino acid sequence of the peptide.

Transmembrane domain-connected peptide 14 ["TrM-
10 peptide 14"; LTACLLTLLLYRVGLVRGEKARKGK (SEQ ID NO: 28)]
is peptide 14 that is linked to the native transmembrane
domain of IGF-IIR. It has been demonstrated that, in
phospholipid vesicles, TrM-peptide 14 can interact with
G_{i2} in a manner more similar to that of the native holo
15 receptor than can peptide 14 (Okamoto et al., 1990). In
one of the experiments shown in Fig.1B, 0.3 nM G_{i2}
reconstituted in phospholipid vesicles with 100nM TrM-
peptide 14 was exposed to various concentrations of ACG_{i2},
and GTPγS binding was measured (◊). The basal fractional
20 GTPγS binding to G_{i2} in this experiment was 0.39 (5 min^{-1}), and the 100% binding level was 0.88 (5 min^{-1}). ACG_{i2}
completely blocked the stimulatory effect of 100 nM TrM-
peptide 14, with an IC_{50} of 3 μM (Fig. 1B). Thus, even
where the context more closely approximates the native
25 environment of the G protein than in the experiment shown
in Fig.1A, ACG_{i2} effectively blocks the stimulatory effect
of the couplone on G_{i2} with the same potency and efficacy
as observed in the latter experiment.

The effect of ACG_{i2} on βIII-2-induced G_s
30 activation is shown in Fig.1B (□). GTPγS binding was
measured following incubation of solubilized G_s at 10nM
with 1μM βIII-2 (RRSSKFCLKEHKALK; SEQ ID NO: 29) in the
presence of ACG_{i2} for 10 min. The basal fractional GTPγS
binding to G_s was 0.30 (10 min^{-1}), and 100% binding was
35 0.60 (10 min^{-1}). ACG_{i2} had no effect at 1μM. Unlike

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inhibition of stimulation of G_{i2}, which reaches 100% at $3\mu M$ ACG_{i2}, the maximal inhibition of $\beta III-2$-stimulated G_s by ACG_{i2} was only 40%, reflecting the fact that the G_s anticouplone is not identical to ACG_{i2}, and so the latter

5 peptide binds to the G_s-coupled receptor relatively inefficiently. At higher concentrations, ACG_{i2} proved to have a positive rather than inhibitory effect on G_s stimulation.

The region of $G_s\alpha$ that is comparable to the ACG_{i2}

10 region of $G_{i2}\alpha$ in topographical structure was synthesized; this region is defined by Asn384-Tyr398 of $G_s\alpha$ (NDCRDIIQRMHLRQY; SEQ ID NO: 12). At concentrations ranging up to 100 μM, this peptide (ACG_s) had no effect at all on peptide 14-induced G_{i2} activation, again

15 reflecting the specificity of each anticouplone (data not shown).

Peptide 14 is capable of activating G_{i1} and G_{i3} as well as G_{i2} (Okamoto et al., 1990). The effect of ACG_{i2} on peptide 14-induced activation of these G_i proteins was

20 examined as follows: 10 nM G_{i1} ($\square$) or G_{i3} ($\lozenge$) was incubated with 30 μM peptide 14 in the presence of various concentrations of ACG_{i2} for 4 min, and GTPγS binding was measured. The fractional GTPγS binding to G_{i1} or G_{i3} at 0% was (in 4 min^{-1}) 0.38 or 0.30, and that at

25 100% was 0.54 or 0.63, respectively. Surprisingly, ACG_{i2} had relatively little effect on G_{i1} activation (Fig. 1C). Even high concentrations ($\geq$ 30 μM) of ACG_{i2} maximally inhibited G_{i1} activation by only 30%. The G_{i1} preparation used had trace contamination by G_o, as described (Katada

30 et al., *FEBS Lett.* 213:353-358 (1987). Since the region of $G_{i1}\alpha$ comparable to ACG_{i2} has a sequence identical to ACG_{i2}, as mentioned above, it appears this region on $G_{i1}\alpha$ does not possess the same function as the identical region on $G_{i2}\alpha$.

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Fig. 1C also shows the effect of ACG_{i2} on peptide 14-induced G_{i3} activation. ACG_{i2} maximally inhibited G_{i3} activation by $\approx 60\%$. This suggests that ACG_{i2} has an antagonizing effect on G_i protein activation in the order

5 of $G_{i2} > G_{i3} > G_{i1}$. These results indicate that the ACG_{i2} peptide blocks G_{i2} activation in a selective manner.

The MIII peptide is a G_i/G_o-activator region in the third intracellular loop of the G-coupled receptor termed M_4AChR (Okamoto et al., J. Biol. Chem. 267:8342-

10 8346, 1992). This peptide has the sequence RNQVRKKRQMAARERKVTR (SEQ ID NO: 30). In the experiments illustrated in Fig. 1D, 10 nM G_o ($\square$) or G_{i2} ($\lozenge$) was incubated with 1 μM MIII for 2 or 5 min, respectively, and GTPγS binding was measured in the same manner as in

15 Fig. 1A. The fractional GTPγS binding to G_o at 0% and 100% was 0.36 and 0.86 (2 min^{-1}), respectively, and that to G_{i2} at 0% and 100% was 0.30 and 0.96 (5 min^{-1}), respectively. MIII-induced G_{i2} activation was found to be attenuated by ACG_{i2} in a dose-dependent manner, with an

20 IC_{50} of 10 μM (Fig. 1D). ACG_{i2} can therefore inhibit G_{i2} activation caused by the effector peptides of m_4AChR and IGF-IIR with similar potencies. In contrast, ACG_{i2} had no effect on MIII-induced G_o activation at $\leq$ 10 μM, and its effect on MIII-induced G_o activation at higher

25 concentrations ($IC_{50} \approx 30$ μM) was significantly (≈ 3 times) less potent than its effect on MIII-induced G_{i2} activation. This provides strong evidence that ACG_{i2} acts on G proteins rather than on receptor peptides.

To verify the notion that ACG_{i2} acts on G_{i2}

30 itself, and not on receptor sequences, two series of experiments were performed. First, the dose-response curve for peptide 14 action on G_{i2} in the presence of various concentrations of ACG_{i2} was generated (Fig. 2A). Following incubation of 10 nM G_{i2} with various

35 concentrations of peptide 14 in the absence ($\square$) or

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presence of 10 μM ($\diamond$) or 30 μM ($\square$) ACG_{i2} for 5 min, GTPγS
binding was assayed; fractional GTPγS binding to G_{i2} is
indicated in the figure. As the concentration of ACG_{i2}
increased, the efficacy of peptide 14 action was found to
5   be decreased. This suggests that ACG_{i2} does not compete
with G_{i2} for peptide 14.

The second series of experiments investigated the
effect of ACG_{i2} on lower concentrations of G_{i2} that had
been activated by peptide 14 to the same extent
10 (Fig. 2B). 1nM G_{i2} was incubated for 5 min with 3 μM
peptide 14 in the presence of various concentrations of
ACG_{i2}, and GTPγS binding was measured. The fractional
GTPγS binding at 0% and 100% in this experiment was 0.40
and 0.55 (5 min^{-1}), respectively. As shown in Fig. 2B,
15 the IC_{50} of the ACG_{i2} effect on the activation of 1 nM G_{i2}
was $\approx 3\mu$M, which is identical to the IC_{50} observed with
10nM G_{i2}. If one assumes that ACG_{i2} inhibits G_{i2}
activation by acting on receptor sequences, the
antagonizing effect of ACG_{i2} on smaller amounts of G_{i2} at
20 a similar intensity of peptide 14 stimulation would be
observed at lower concentrations of ACG_{i2}, resulting in a
lower IC_{50}. Conversely, if ACG_{i2} acts on G_{i2}, the potency
of the ACG_{i2} effect on smaller compared to larger amounts
of G_{i2} would not be altered, because the affinity of G_{i2}
25 for ACG_{i2} would presumably determine the potency of ACG_{i2}.
Therefore, the present results suggest that ACG_{i2} inhibits
peptide 14/G_{i2} coupling by acting on G_{i2} with constant
affinity.

By examining the relationship between the
30 structure of ACG_{i2} and its function in the inhibition
assay described above, it was possible to determine the
primary structure essential for the inhibitory action of
ACG_{i2}. In the experiments shown in Figs. 3a-3c, G_{i2} at 10
nM was incubated with 30 μM peptide 14 in the presence of
35 various concentrations of ACG_{i2} or one of its structural

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variants, and GTPγS binding to G_{i2} was measured. The
sequence of each peptide is indicated above the graphs (a
bar indicates the same sequence as intact ACG_{i2}). The 0%
and the 100% increases, which correspond to the basal and
5   peptide 14-stimulated fractional GTPγS binding to G_{i2},
were similar to those described in Fig. 1A. These groups
can be practically classified according to the inhibition
observed at 10 μM. The high potency peptides of the
first group (Fig. 3A) display $\geq$ 80% inhibition at 10μM
10   concentration; these peptides are the A_2 variant
(DAVT**A**VIIKNNLKDC; SEQ ID NO: 21), in which the Asp at
position five is replaced by Ala, and the A_5 variant
(DAVTDVIIKNNLK**A**C; SEQ ID NO: 24), in which the Asp at
position 14 is replaced by Ala. The second, low potency
15   group (Fig. 3B) displays $\leq$ 30% inhibition at 10 μM, and
is made up of the A_1 variant (**A**AVTDVIIKNNLKDC; SEQ ID
NO: 20), in which the Asp at position 1 is replaced by
Ala; the A_3 variant (DAVTDVIIK**A**NLKDC; SEQ ID NO: 22), in
which the Asn at position 10 is replaced with Ala; the p9
20   variant (DAVTDVIIK; SEQ ID NO: 25), in which the carboxy-
terminal 6 residues of ACG_{i2} are deleted; and the del C
variant (DAVTDVIIKNNLKD; SEQ ID NO: 16), in which the C-
terminal Cys is deleted from ACG_{i2}. The third,
intermediate potency group (Fig. 3C) consists of the A_4
25   variant (DAVTDVIIKN**A**LKDC; SEQ ID NO: 23), in which the
Asn at position 11 is replaced by Ala; and the p11
variant (DVIIKNNLKDC; SEQ ID NO: 26), in which the amino-
terminal 4 residues are deleted.
      These results suggest that the extreme amino-
30   terminal Asp, the Asn residues at positions 10 and 11,
and the carboxy-terminal Cys are essential for the
inhibitory activity of ACG_{i2}, and that, since both the
amino-terminal residue and the carboxy-terminal residue
of ACG_{i2} are required, the segment of $G_{i2}\alpha$ represented by
35   ACG_{i2} corresponds to the minimal anticouplone domain of

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$G_{i2}\alpha$. In contrast, the Glu residues at positions 2 and 14
appear to be dispensable. One can conclude from this
information that, contrary to the primary hypothesis used
for the initial search that anionic residues on $G_{i2}\alpha$ would
5 interact with the basic residues of peptide 14, all of
the negatively charged residues are unlikely to be
oriented facing the basic residues of peptide 14 or MIII,
or at least that such a matchup of anionic and basic
residues is not of critical importance at some of the
10 sites. It remains unclear how the Cys^{352} residue of ACG_{i2}
plays a key role.

To determine whether ACG_{i2} uncouples G_{i2} from the
signals of intact receptors as well as from the signals
of their effector peptides, the effects of ACG_{i2} in mAChR-
15 G_{i2} reconstituted phospholipid vesicles was studied
(Fig. 4). In these vesicles, the basal value of rate
constant $kapp$ of GTPγS binding to G_{i2} in the absence of
acetylcholine was 0.112 ± 0.005 (min-1); 10 mM
acetylcholine (for 20 min at 30°C) increased the k_{app}
20 value to 0.286 ± 0.082. The magnitude of the
acetylcholine effect is similar to that previously
reported (Kurose et al., J. Biol. Chem. 261:6423-6428,
1986). When 10 mM acetylcholine was incubated with the
vesicles in the presence of 30 μM ACG_{i2}, the k_{app} value of
25 GTPγS binding to G_{i2} was found to be 0.157 ± 0.013. This
indicates that the G_{i2} activation induced by acetylcholine
in AChR-G_{i2} vesicles was ≈ 75% inhibited by ACG_{i2}. ACG_{i2}-
induced inhibition was saturated at 30 μM (data not
shown). ACG_{i2} had no significant effect on basal G_{i2}
30 activity in the absence of acetylcholine (Fig. 4).

A model illustrating the possible way in which
ACG_{i2} blocks the interaction of G_{i2} and Peptide 14 is
shown in Fig. 5. It is postulated that the anticouplone
portion of $G_{i2}\alpha$ acts on a hypothetical second site of $G_{i2}\alpha$
35 itself, thereby stabilizing $G_{i2}\alpha$ in the unstimulated state

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(Fig. 5, left). Peptide 14, which has the sequence of
the effector (couplone) region of IGF-IIR, binds to the
contact site on $G_{i2}\alpha$, resulting in a conformational change
in $G_{i2}\alpha$ that diminishes the accessibility of the
5 anticouplone region of $G_{i2}\alpha$ to the hypothetical second
site on $G_{i2}\alpha$; this leads to a decrease in binding of the
two regions of $G_{i2}\alpha$ and a concomitant activation of the G
protein (Fig. 5, upper right). Addition of ACG_{i2} peptide
causes the hypothetical second site on $G_{i2}\alpha$ to be occupied
10 regardless of the presence or absence of Peptide 14,
thereby stabilizing $G_{i2}\alpha$ in the inactive state in a manner
that is independent of (and so does not compete with) $G_{i2}\alpha$
binding to Peptide 14. Although the receptor-contact
site is suggested to be different than the ACG_{i2} region of
15 $G_{i2}\alpha$ in this figure, it should be emphasized that they are
not necessarily different. Furthermore, it is noted that
in the actual intracellular environment, there are other
factors (e.g., nucleotide-binding status, GTP hydrolytic
activity, and association with $G\beta\gamma$) that critically
20 affect the equilibrium of each step. Because ACG_{i2} has
different effects on the various G proteins (see above),
it is unlikely that the hypothetical second site resides
on $G\beta\gamma$ (which is identical for all of the G proteins)
rather than $G\alpha$ (which is G-protein-specific).

25 <u>Cell-based Assays</u>

    <u>*Inhibition of somatostatin receptor function.*</u> It
is known that somatostatin induces K^+ channel activation
in a manner sensitive to pertussis toxin in human growth
hormone-secreting tumors (Yamashita et al., Am. J.
30 Physiol. 253:E28-E32, 1987). The effect of each of ACG_{i2},
ACG_{i3}, ACG_s, ACG_{o1}, and ACG_{o2} on somatostatin-induced K^+
currents in human pituitary cells was measured using the
patch clamp technique, as described below.

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Three human growth hormone-secreting pituitary
adenomas were obtained at transsphenoidal surgery. The
tissue was minced into small pieces (less than 1 mm in
diameter) and was treated with 1000 units/ml dispase
5 (Wako, Japan). These dissociated cells ($\approx 10^5$ cells/ml)
were seeded onto 35-mm culture dishes. The monolayer
cells were cultured at 37°C under 5% CO_2 in humidified
air in Delbecco's modified Eagle's medium (DMEM)
containing 10% heat-inactivated FCS.
10 Electrophysiological experiments were done within 4 weeks
after cell preparation.

A whole cell variation of the patch electrode
voltage clamp technique was used to record membrane
currents (Hamill et al., Pflüger Arch. 391:85-100, 1987).
15 The patch electrode solution contained 95 mM potassium
aspartate, 47.5 mM KCl, 1 mM $MgCl_2$, 5.0 mM EGTA
(potassium salt), 10 mM HEPES/KOH (pH 7.2), 2 mM ATP and
0.1 mM GTP. One of the anticouplone peptides, dissolved
in distilled water, was mixed with the patch electrode
20 solution to a final concentration of 100 μM, and was
introduced into the interior of a pituitary cell in
accordance with the published method. The extracellular
medium, composed of 125 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$,
2.5 mM $CaCl_2$, and 10 mM HEPES/NaOH (pH 7.4), was
25 continuously perfused around the test cell during
recording, using a peristaltic pump, and 10^{-7}M
somatostatin was introduced by changing the perfused
medium. The liquid junction potential between the
extracellular solution and the internal solution was
30 directly measured using a 3 M KCl electrode as a
reference. The value of the liquid junction potential (-
8 mV) was corrected in each experiment. Glass
capillaries of 1.5 mm diameter with a filament were used
to fabricate an electrode for the whole cell clamp. They
35 were coated with Sylgard and fire-polished before use.

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The patch electrode resistance used in these studies was in the range of 5 to 8 MΩ. The seal resistance was over several tens of GΩ. A List EPC-7 amplifier was used for the membrane current and potential. Application of

5   voltage or current pulse, data acquisition, and analysis were done with a Gateway 2000 computer using the pCLAMP program (Axon Instruments, CA). All experiments were performed at room temperature (22-25°C).

The intracellular solution that filled the inside

10 of the pipet portion of the apparatus contained one of the following anticouplone peptides at 100 μM: ACG_{i2}, ACG_{i3}, ACG_s, ACG_{o1}, or ACG_{o2}. The exterior of the cell was bathed with the extracellular solution containing 100 nM somatostatin while the outward K^+ current was

15 monitored. In the absence of anticouplone peptides, somatostatin induces approximately 20 pA K^+ current over baseline. This somatostatin-induced current level is not affected by the presence of 100 μM ACG_{i3}, ACG_s, ACG_{o1}, or ACG_{o2}. ACG_{i2}, however, abolished the somatostatin-induced

20 increase in K^+ current, reducing the measured current to the baseline level observed in the absence of somatostatin. These data suggest that G_{i2}, but not G_{i3}, G_s, G_{o1}, or G_{o2}, is involved in the somatostatin receptor/K^+ channel linkage, and that ACG_{i2} can

25 specifically inhibit the ability of the somatostatin receptor to act on ion channels. Since this function of the somatostatin receptor on endocrine cells is related to the inhibition of hormone secretion by such cells, modulation of this receptor/G protein system by ACG_{i2}

30 permits the control of hormone secretion from endocrine cells.

Inhibition of cellular growth. $G_{i2}\alpha$ has been shown to be an oncogene in certain human tumors (Lyons et al, Science 249:655-659, 1990). The effect of the

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peptide ACG_{i2} on the growth of CHO cells was measured as follows:

CHO cells were grown in Ham F12 medium (GIBCO) plus 10% fetal calf serum (FCS). Once they reached at

5   least 80% confluency, the cells were incubated with Ham F12 plus 0.5% FCS for 24 hours. They were then incubated for an additional 24 hours with Ham F12 plus 10% FCS in the presence of BUdR and various concentrations of ACG_{i2}. The cells were then fixed and the BUdR incorporated into

10 their nuclei was labeled in accordance with the instructions included with the cell proliferation detection kit (RPN.210, Amersham). Fig. 6 shows the relative extent of BUdR incorporation into cells in the presence of no ACG_{i2} (top photograph), $10\mu M$ ACG_{i2} (center

15 photograph), or 100 μM ACG_{i2} (bottom photograph), wherein a labelled nucleus signifies a proliferating cell. It can be seen that FCS-induced proliferation of CHO cells is inhibited by ACG_{i2} in a dose-dependent fashion, with 10 μM ACG_{i2} producing significant inhibition and 100 μM

20 inhibiting labelling almost completely. This suggests that ACG_{i2} may have value as an anti-growth or anti-cancer reagent.

Inhibition of TGFβ-induced matrix formation. The secretion of matrix proteins (in particular, fibronectin)

25 by kidney, lung and liver cells may lead to sclerosis of those organs. Transforming growth factor-β (TGFβ), which stimulates the secretion of fibronectin, may therefore contribute to such sclerosis. Since the TGFβ receptor is a G_{o2}-coupled receptor, the ability of ACG_{o2} to inhibit

30 the TGFβ-induced stimulation of fibronectin secretion by NRK (rat renal fibroblast) cells (Ignotz and Massagué, J. Biol. Chem. 261:4337-4345, 1986) was assayed.

NRK cells were cultured in RPMI 1640 supplemented with 5% FCS in a CO_2-incubator at 37°C. The medium was

35 replaced every two days. Cells were regularly

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subcultured by trypsin-EDTA before reaching confluency.
In the experiments described below, the cells were grown
in monolayer to confluency in 6-well culture plates
(Corning, NY), then incubated for 12 hours with RPMI 1640

5 plus 0.5% FCS, and for a further 12 hours with RPMI 1640
plus 0.5% FCS in the presence or absence of 50 pM TGFβ
(R & D System, Minneapolis, MN), to which was added
100 μM ACG_{i2}, ACG_s, ACG_{o2}, or ACG_q. The medium from each
well was collected and frozen at -20_oC until assay.

10 Fibronectin secreted into each sample of medium was
assessed by immunoblot as follows:

Each medium sample was mixed with an equal volume
of 2x concentrated Laemmli buffer (Laemmli, Nature
227:680-685, 1970), and boiled at 90°C for 5 min.

15 Proteins were separated by electrophoresis on 7.5% SDS-
polyacrylamide gel, and then transferred to a
polyvinylidene difluoride sheet (Immobilon-P, Millipore)
by electroelution at 0-4°C with constant voltage of 75 V
for 8 hours. After transfer, the sheet was incubated

20 with PBS containing 5% bovine serum albumin and 2% skim
milk (Defco) overnight at room temperature to block
nonspecific protein binding. The sheet was then
incubated with PBS containing 0.1% bovine serum albumin
and an anti-fibronectin antibody (Chemicon International,

25 CA) at a final concentration of 0.5 mg/ml for 3 hours at
room temperature. After washing with ice-cold PBS/0.05%
Tween 20 three times, the sheet was incubated with
0.04 units/ml of a horseradish peroxidase-conjugated
anti-rabbit IgG (Sigma, A-8257) in PBS/0.1% bovine serum

30 albumin for 3 hours at room temperature. The sheet was
washed six times with ice-cold PBS/0.05% Tween 20,
incubated with ECL (Enhanced ChemiLuminescence) solution
(Amersham, RPN 2101) according to instructions for 1 min,
and exposed on Kodak XAR5 film for 40-60 seconds. As

35 shown in Fig. 7, a significant amount of fibronectin is

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secreted by the cells exposed to TGFβ, whether or not any
of ACG_{i2}, ACG_s, or ACG_q is present in the culture medium.
In contrast, those cells exposed to TGFβ in the presence
of ACG_{o2} secrete relatively little fibronectin. This

5 indicates that ACG_{o2} can inhibit the TGFβ-induced
secretion of fibronectin by these cells, suggesting that
ACG_{o2} has use as a therapeutic for the inhibition of
organosclerosis, as well as of TGFβ activity in general.

   Inhibition of T cell receptor function with ACG_q.

10 T cell receptor stimulation by monoclonal antibody OKT^3
in Jurkat cells, a line of human T cells, induces
measurable polyphosphoinositide (PI) turnover. 10^7 cells
were labelled with 5μCi/ml [^{3}H]myoinositol in inositol-
free RPMI1640 for 12 hours. After incubating the cells

15 with 500 μM ACG_q, samples containing 5×10^5 cells were then
exposed to the monoclonal antibody OKT_3 (Ortho) at 1:100
dilution for 5 min. Perchloric acid was added to a final
concentration of 10% to lyse the cells and terminate the
reaction. Cell lysates were collected into tubes and

20 brought to pH$\rangle$8 by the addition of KOH. Following
centrifugation of the lysates, the supernatants were
passed over a DOWX anion exchange column (Muromachi
Chemicals, Japan) to separate inositol polyphosphates
(IPs) from other inositol metabolites, and the amount of

25 [^{3}H] label in the inositol trisphosphate (IP_3) fraction
assessed. Cells exposed to OKT_3 incorporated [^{3}H] into IP
to a level 157% that of the control cells (cells not
exposed to OKT_3), reflecting the stimulatory effect OKT_3
has on PI turnover in T cells. When the cells pretreated

30 with 500 μM ACG_q were stimulated with OKT_3, the level of
[^{3}H] incorporation into IP_3 was only 112% that of the
unstimulated control cells; in contrast, pretreatment
with 500 μM ACG_{i2} did not inhibit stimulation of the cells
by OKT_3. These results indicate that ACG_q, can block the

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function of the T cell receptor, suggesting a use of this anticouplone peptide as an immunosuppressant.

In vivo Assay of Anticouplone Peptides

Myopathic syrian hamsters BIO 53.58 spontaneously
5   develop fibrosis of the heart muscle (i.e., development
of extraneous collagen fibers that eventually interfere
with muscle function) by about 16 weeks of age, and as
such are used as models for the human disease
cardiomyopathy. In the experiment reported herein, 10-
10 week-old BIO 53.58 hamsters received intraperitoneal
injections with 5 μl of 10 mM ACG_q, 10 mM ACG_{i2}, or
physiological saline once a week for 6 weeks. The
animals were then decapitated and their hearts were
excised, fixed in 10% formaldehyde solution, and cut
15 horizontally at the level of the papillary muscle to make
sections for light microscopy. The muscle sections were
stained with sirius red (which specifically stains
collagen), and evaluated under the microscope. In each
photomicrograph shown in Fig. 8, the right ventricular
20 free wall is located at the left of the photomicrograph
and the interventricular septum is on the right. As
shown in Fig. 8, top panel, the control hamsters
exhibited a significant amount of heart muscle fibrosis
(indicated by darkly stained lines). In contrast, the
25 animals treated with ACG_{i2} for six weeks had remarkably
diminished collagen fiber formation (Fig. 8, bottom
panel). Hamsters which received ACG_q had undiminished,
control levels of fibrosis (data not shown).

Use

30   Each of the anticouplone peptides of the invention
may be used as a therapeutic for appropriate indications.
ACG_{i2} may be used where inhibition of G_{i1}- or G_{i2}-coupled
receptor function is desired: for example, to treat

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neuromuscular diseases and cardiac arrythmia by
inhibiting muscarinic acetylcholine receptors; to
alleviate dwarfism by inhibiting stimulation of
somatostatin receptors on pituitary cells; to block
5 cancerous cell proliferation (or other undesired cell
growth, such as cardiomyopathy) by inhibiting the G_{i2}
oncogene; to treat organofibrosis by inhibiting TGFβ
receptors; and to treat malignant hypercalcemia by
inhibiting the parathyroid/parathyroid-related protein
10 (PTH/PTHrp) receptor. ACG_{i3} would be useful for treatment
of conditions related to organization and polarization of
cells and cellular structures, as during organ
development.

    ACG_{o1} will inhibit stimulation of the nerve growth
15 factor receptor (NGFR), the activity of which is thought
to affect neuronal growth. ACG_{o2} is useful for inhibiting
the TGFβ-induced stimulation of fibronectin secretion
which leads to fibrosis and sclerosis of organs such as
kidney, lung and liver. Inhibition of TGFβ is also
20 useful in controlling the generation of cancer clones and
in treating glomeronephritis. In addition, ACG_{o2} can be
used to prevent cancer cell growth attributable to
abnormal stimulation of the cells by a mutant G_{o2}
oncogene.

25   Because G_q is thought to be the G protein to which
the T cell receptor is coupled, ACG_q would be useful for
inhibiting the function of this receptor, and thus the
activation of T cells. This anticouplone therefore may
be used as an immunosuppressant, such as in organ and
30 bone marrow transplant recipients, and to treat
autoimmune disease. ACG_q also has potential as a
treatment for AIDS, by inhibiting the abnormal signalling
that results from binding of HIV gp120 to CD4 on the T
cell surface. In addition, ACG_q, which inhibits

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signalling through the angiotensin receptor, may be used
as a treatment for hypertension.

     G_s-coupled receptors include the glucagon
receptor, the β_2AR, and the PTH/PTHrP receptor. ACG_s
5 could therefore be utilized as a treatment for diabetes,
hypertension, and malignant hypercalcemia. Because a
mutant form of G_s acts constitutively in some pituitary
tumors, ACG_s could be used to treat such pituitary tumor-
related conditions as giantism and secondary menopause.
10   The treatment protocol to be used for any one of
the above indications could be readily determined by one
of ordinary skill in the art of pharmacology, given the
teachings set forth above. The peptides of the invention
may be administered by a standard method such as
15 intravenous, intraperitoneal, intramuscular, or
subcutaneous injection, and may be administered in a
single dose, several doses spread over time, or a
continuous infusion. The dosage will vary with the age
and condition of the patient and the particular condition
20 being treated, but is expected to be in the range of
$0.5\mu g/kg/day$ to 50 mg/kg/day. A dosage which results in
a concentration at the target site of approximately 10-
$100\mu M$ would be an effective dose consistent with the
experimental results provided above. Incorporating the
25 peptides into micelles or liposomes may improve delivery
into cells.

Other Embodiments

     Other embodiments are within the claims set forth
below. For instance, many other receptors are known or
30 believed to be G-coupled receptors, based in part on the
their distinctive arrangement of multiple transmembrane
domains known to be typical of G-coupled receptors;
inappropriate signalling through these receptors can be
controlled by the peptides of the invention. An example

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is the thyroid-stimulating hormone (TSH) receptor,
overstimulation of which (e.g., by a receptor-specific
antibody) leads to hyperthyroidism, or Basedow's disease.
This receptor is probably a G_s-coupled receptor. Another
5 example is the G protein-coupled insulin receptor, which
may be overstimulated in a patient with an insulin-
secreting tumor.

       As described above, some amino acid residues
within the defined anticouplone sequences can be replaced
10 with other residues, with no significant loss of
biological activity. For example, in ACG_{i2}, an Asp→Ala
substitution at position 5 or 14 may be made with little
or no loss of activity, while similar nonconservative
substitutions at positions 1, 10, and 11 resulted in a
15 significant loss of activity in the in vitro assay used.
In general, conservative substitutions in which the
relative charge of the residue occupying the substituted
position does not vary (i.e., replacing an anionic
residue with another anionic residue, or a hydrophobic
20 residue with another hydrophobic residue, etc.) will have
less effect on the biological activity of the resulting
peptide than will nonconservative substitutions. While
deletion of some residues may well not affect biological
activity significantly, it is known from the work
25 described above that the residues in both the first and
the last (15th) positions of ACG_{i2} are important for
activity of that anticouplone. Variants of the
anticouplone peptides utilized in the above experiments
can be readily made by one of ordinary skill in the art
30 of peptide synthesis, using standard methods of automated
synthesis; each such peptide can then be tested in one or
more of the biological assays described above. The in
vitro, cell-free assays are particularly suited to
testing large numbers of such peptides quickly and
35 efficiently, permitting one to produce and evaluate

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hundreds of peptide variants without undue
experimentation. Thus, the invention is meant to include
any peptide which (a) has at least 70% sequence identity
with one of the anticouplone peptides described above

5   (preferably at least 85%), and (b) has at least 80% of
the biological activity of the anticouplone peptide which
it most closely resembles, in any of the biological
assays described above in which that anticouplone peptide
has been shown to be active. Of course, a peptide which

10 includes the sequence of one of the anticouplones
described above, plus additional sequence (including but
not limited to sequence derived from the G-protein of
which the anticouplone is a part), would be expected to
have undiminished biological activity compared to the

15 anticouplone itself, provided that the peptide is not too
bulky or too highly charged to pass through the cellular
membrane as readily as does the anticouplone peptide.
Again, such longer peptides can be easily prepared and
tested as described. Examples of such longer peptides

20 are presented above as "alternative" anticouplone
peptides, shown as SEQ ID NOs: 4, 6, 9, 11, 13, and 15.
        Also within the invention are analogs of the above
peptides, in which one or more peptide bonds have been
replaced with an alternative type of covalent bond (a

25 "peptide mimetic") which is not susceptible to cleavage
by peptidases. While the *in vivo* results described above
suggest that proteolytic degradation of the peptides
following injection into the subject animal may not be a
problem, it may be that for certain of the peptides,

30 replacement of a particularly sensitive peptide bond with
a noncleavable peptide mimetic will make the resulting
compound more stable and thus more useful as a
therapeutic. Such mimetics, and methods of incorporating
them into polypeptides, are well known in the art.

35 Similarly, the replacement of an L-amino acid residue of

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the anticouplone peptide with the corresponding D-amino acid residue is a standard way of rendering the polypeptide less sensitive to proteolysis. Also useful in this regard are amino-terminal blocking groups such as

5 t-butyloxycarbonyl, acetyl, ethyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4-dinitrophenyl. Blocking the charged amino- and carboxy-

10 termini of the peptides would have the additional benefit of enhancing passage of the peptide through the hydrophobic cellular membrane and into the cell.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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    (ii) TITLE OF INVENTION: REGULATOR REGIONS OF G PROTEINS

    (iii) NUMBER OF SEQUENCES: 34

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        (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
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```
        (i) SEQUENCE CHARACTERISTICS:

                (A) LENGTH:                 15
                (B) TYPE:                    amino acid
                (C) STRANDEDNESS:
                (D) TOPOLOGY:                linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:


Asp Xaa Xaa Xaa Xaa Val Ile Ile Lys Asn Asn Leu Xaa Xaa Cys
 1               5                   10                  15


(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:    2:

        (i) SEQUENCE CHARACTERISTICS:

                (A) LENGTH:                 15
                (B) TYPE:                    amino acid
                (C) STRANDEDNESS:
                (D) TOPOLOGY:                linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:


Asp Ala Val Thr Xaa Val Ile Ile Lys Asn Asn Leu Lys Xaa Cys
 1               5                   10                  15


(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:    3:

        (i) SEQUENCE CHARACTERISTICS:

                (A) LENGTH:                 15
                (B) TYPE:                    amino acid
                (C) STRANDEDNESS:
                (D) TOPOLOGY:                linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:


Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Asp Cys
 1               5                   10                  15


(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:    4:

        (i) SEQUENCE CHARACTERISTICS:

                (A) LENGTH:                 24
                (B) TYPE:                    amino acid
                (C) STRANDEDNESS:
                (D) TOPOLOGY:                linear

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:


Asp Thr Lys Asn Val Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile
 1               5                   10                  15

Ile Lys Asn Asn Leu Lys Asp Cys
   20
```

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 15
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Glu Cys
  1   5   10   15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 24
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Asp Thr Lys Asn Val Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile
  1   5   10   .   15

Ile Lys Asn Asn Leu Lys Glu Cys
            20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 15
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asp Ala Val Thr Xaa Ile Ile Ile Ala Xaa Asn Leu Arg Xaa Cys
  1   5   10   15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 15
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

- 33 -

```
Asp Ala Val Thr Asp Ile Ile Ile Ala Asn Asn Leu Arg Gly Cys
 1               5                   10                      15
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 22
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Asn Asn Ile Gln Val Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala
 1               5                   10                      15

Asn Asn Leu Arg Gly Cys
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 15
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Asp Ala Val Thr Asp Ile Ile Ile Ala Lys Asn Leu Arg Gly Cys
 1               5                   10                      15
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 22
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Asn Asn Ile Gln Phe Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala
 1               5                   10                      15

Lys Asn Leu Arg Gly Cys
            20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 15
        (B) TYPE: amino acid

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```
        (C) STRANDEDNESS:
        (D) TOPOLOGY:                    linear


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:


Asn Asp Cys Arg Asp Ile Ile Gln Arg Met His Leu Arg Gln Tyr
 1               5               10                      15


(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:      13:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH:                      24
        (B) TYPE:                        amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY:                    linear


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:


Asp Thr Glu Asn Ile Arg Arg Val Phe Asn Asp Cys Arg Asp Ile Ile
 1               5               10                      15

Gln Arg Met His Leu Arg Gln Tyr
                20


(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:      14:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH:                      15
        (B) TYPE:                        amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY:                    linear


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:


Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr
 1               5               10                      15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:      15:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH:                      24
        (B) TYPE:                        amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY:                    linear


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:


Asp Thr Glu Asn Ile Arg Phe Val Phe Ala Ala Val Lys Asp Thr Ile
 1               5               10                      15

Leu Gln Leu Asn Leu Lys Glu Tyr
                20
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 14
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Asp
1   5   10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 17:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 14
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Glu
1   5   10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 18:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 14
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Glu Ala Ala Ser Tyr Ile Gln Ser Lys Phe Glu Lys Leu Asn
1   5   10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 19:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 10
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Lys Asn Asn Leu Lys Asp Cys Gly Leu Phe
1   5   10

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 20:

   (i) SEQUENCE CHARACTERISTICS:

      (A) LENGTH: 15
      (B) TYPE: amino acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Ala Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Asp Cys
 1   5   10   15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 21:

   (i) SEQUENCE CHARACTERISTICS:

      (A) LENGTH: 15
      (B) TYPE: amino acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Asp Ala Val Thr Ala Val Ile Ile Lys Asn Asn Leu Lys Asp Cys
 1   5   10   15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 22:

   (i) SEQUENCE CHARACTERISTICS:

      (A) LENGTH: 15
      (B) TYPE: amino acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Asp Ala Val Thr Asp Val Ile Ile Lys Ala Asn Leu Lys Asp Cys
 1   5   10   15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 23:

   (i) SEQUENCE CHARACTERISTICS:

      (A) LENGTH: 15
      (B) TYPE: amino acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Ala Leu Lys Asp Cys
 1   5   10   15

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 24:

    (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 15
            (B) TYPE: amino acid
            (C) STRANDEDNESS:
            (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Ala Cys
 1   5   10   15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 25:

    (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 9
            (B) TYPE: amino acid
            (C) STRANDEDNESS:
            (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Asp Ala Val Thr Asp Val Ile Ile Lys
 1   5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 26:

    (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 11
            (B) TYPE: amino acid
            (C) STRANDEDNESS:
            (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Asp Val Ile Ile Lys Asn Asn Leu Lys Asp Cys
 1   5   10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 27:

    (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 14
            (B) TYPE: amino acid
            (C) STRANDEDNESS:
            (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Arg Val Gly Leu Val Arg Gly Glu Lys Ala Arg Lys Gly Lys
 1   5   10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 28:

    (i) SEQUENCE CHARACTERISTICS:

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```
                    (A) LENGTH:                    25
                    (B) TYPE:                      amino acid
                    (C) STRANDEDNESS:
                    (D) TOPOLOGY:                  linear

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:


Leu Thr Ala Cys Leu Leu Thr Leu Leu Leu Tyr Arg Val Gly Leu Val
1               5                   10                  15

Arg Gly Glu Lys Ala Arg Lys Gly Lys
            20                  25


(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:      29:

        (i) SEQUENCE CHARACTERISTICS:

                    (A) LENGTH:                    15
                    (B) TYPE:                      amino acid
                    (C) STRANDEDNESS:
                    (D) TOPOLOGY:                  linear

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Arg Arg Ser Ser Lys Phe Cys Leu Lys Glu His Lys Ala Leu Lys
1               5                   10                  15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:      30:

        (i) SEQUENCE CHARACTERISTICS:

                    (A) LENGTH:                    19
                    (B) TYPE:                      amino acid
                    (C) STRANDEDNESS:
                    (D) TOPOLOGY:                  linear

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Arg Asn Gln Val Arg Lys Lys Arg Gln Met Ala Ala Arg Glu Arg Lys
1               5                   10                  15

Val Thr Arg


(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER:      31:

        (i) SEQUENCE CHARACTERISTICS:

                    (A) LENGTH:                    15
                    (B) TYPE:                      amino acid
                    (C) STRANDEDNESS:
                    (D) TOPOLOGY:                  linear

            (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:


Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Xaa Cys
1               5                   10                  15
```

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 32:

  (i) SEQUENCE CHARACTERISTICS:

    (A) LENGTH: 24
    (B) TYPE: amino acid
    (C) STRANDEDNESS:
    (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Asp Thr Lys Asn Val Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile
 1   5   10   15

Ile Lys Asn Asn Leu Lys Xaa Cys
          20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 33:

  (i) SEQUENCE CHARACTERISTICS:

    (A) LENGTH: 15
    (B) TYPE: amino acid
    (C) STRANDEDNESS:
    (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Asp Ala Val Thr Asp Ile Ile Ile Ala Xaa Asn Leu Arg Gly Cys
 1   5   10   15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 34:

  (i) SEQUENCE CHARACTERISTICS:

    (A) LENGTH: 22
    (B) TYPE: amino acid
    (C) STRANDEDNESS:
    (D) TOPOLOGY: linear

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Asn Asn Ile Gln Xaa Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala
 1   5   10   15

Xaa Asn Leu Arg Gly Cys
          20

What is claimed is:

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CLAIMS

1. A molecule comprising the anticouplone sequence of a G protein, wherein said molecule contains no more than 50 amino acid residues.

2. The molecule of claim 1, wherein said molecule
5   contains no more than 30 amino acid residues.

3. The molecule of claim 1, wherein said G protein is a mammalian G protein.

4. The molecule of claim 2, wherein said molecule is a peptide the amino acid sequence of which is
10   essentially identical to said anticouplone.

5. A molecule comprising the amino acid sequence
        DXXXXVIIKNNLXXC (SEQ ID NO: 1),
wherein said molecule contains no more than 40 amino acid residues.

15 6. The molecule of claim 5, wherein said sequence is
        DAVTXVIIKNNLKXC (SEQ ID NO: 2).

7. The molecule of claim 6, wherein said molecule comprises the amino acid sequence
20   DAVTDVIIKNNLKDC (SEQ ID NO: 3).

8. The molecule of claim 6, wherein said molecule comprises the amino acid sequence
        DTKNVQFVFDAVTDVIIKNNLKDC (SEQ ID NO: 4).

9. The molecule of claim 5, wherein said molecule
25 comprises the amino acid sequence
        DAVTDVIIKNNLKEC (SEQ ID NO: 5).

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10.   The molecule of claim 9, wherein said
molecule comprises the amino acid sequence
        DTKNVQFVFDAVTDVIIKNNLKEC (SEQ ID NO: 6).

11.   A molecule comprising the amino acid sequence
5   DAVTXIIIAXNLRXC (SEQ ID NO: 7),
wherein said molecule contains no more than 40 amino acid
residues.

12.   The molecule of claim 11, wherein said
molecule comprises the amino acid sequence
10   DAVTDIIIANNLRGC (SEQ ID NO: 8).

13.   The molecule of claim 11, wherein said
molecule comprises the amino acid sequence
        NNIQVVFDAVTDIIIANNLRGC (SEQ ID NO: 9).

14.   The molecule of claim 11, wherein said
15   molecule comprises the amino acid sequence
        DAVTDIIIAKNLRGC (SEQ ID NO: 10).

15.   The molecule of claim 14, wherein said
molecule comprises the amino acid sequence
        NNIQFVFDAVTDIIIAKNLRGC (SEQ ID NO: 11).

20   16.   A molecule comprising the amino acid sequence
            NDCRDIIQRMHLRQY (SEQ ID NO: 12),
wherein said molecule contains no more than 40 amino acid
residues.

17.   The molecule of claim 16, wherein said
25   molecule comprises the amino acid sequence
        DTENIRRVFNDCRDIIQRMHLRQY (SEQ ID NO: 13).

18.   A molecule comprising the amino acid sequence

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AAVKDTILQLNLKEY (SEQ ID NO: 14),
wherein said molecule contains no more than 40 amino acid
residues.

19.   The molecule of claim 18, wherein said
5   molecule comprises the amino acid sequence
         DTENIRFVFAAVKDTILQLNLKEY (SEQ ID NO: 15).

20.   A method of inhibiting activation of a G
protein by a G-coupled receptor on a cell, said method
comprising contacting said cell with a molecule
10   comprising the anticouplone sequence of said G protein,
said molecule containing no more than 40 amino acid
residues.

21.   The method of claim 20, wherein said G
protein is G_{i1}, G_{i2}, or G_{i3} and said anticouplone sequence
15   is
         DAVTDVIIKNNLKXC (SEQ ID NO: 31) or
         DTKNVQFVFDAVTDVIIKNNLKXC (SEQ ID NO: 32).

22.   The method of claim 21, wherein said G-
coupled receptor is insulin-like growth factor II
20   receptor (IGF-IIR), muscarinic acetylcholine receptor
(mAChR), somatostatin receptor, D_2-dopamine receptor, α_2-
adrenergic receptor, adenosine receptor, thrombin
receptor, or TGFβ receptor.

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23.   The method of claim 20, wherein said G
protein is G_{o1} or G_{o2} and said anticouplone sequence is
DAVTDIIIAXNLRGC (SFQ ID NO: 33) or
NNIQXVFDAVTDIIIAXNLRGC (SEQ ID NO: 34).

5   24.   The method of claim 23, wherein said G-
coupled receptor is transforming growth factor-β receptor
(TGFβR), γ-butyric acid (GABA) receptor, somatostatin
receptor, muscarinic acetylcholine receptor, adenosine
receptor, thrombin receptor, or α_2-adrenergic receptor.

10   25.   The method of claim 20, wherein said G
protein is G_s and said anticouplone sequence is
NDCRDIIQRMHLRQY (SEQ ID NO: 12) or
DTENIRRVFNDCRDIIQRMHLRQY (SEQ ID NO: 13).

26.   The method of claim 25, wherein said G-
15   coupled receptor is β_2-adrenergic receptor, glucagon
receptor, or D_1-dopamine receptor.

27.   The method of claim 20, wherein said G
protein is G_q and said anticouplone sequence is
AAVKDTILQLNLKEY (SEQ ID NO: 14) or
20   DTENIRFVFAAVKDTILQLNLKEY (SEQ ID NO: 15).

28.   The method of claim 27, wherein said G-
coupled receptor is the T cell receptor, PTH/PTHrP
receptor, calcitonin receptor, endothelin receptor,
angiotensin receptor, platelet activating factor
25   receptor, or thromboxane A_2 receptor.
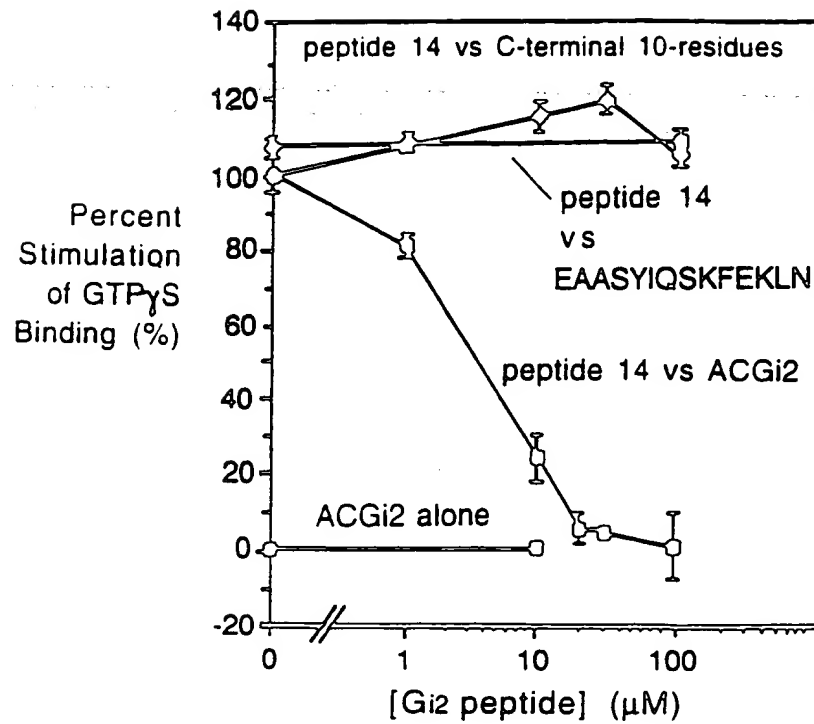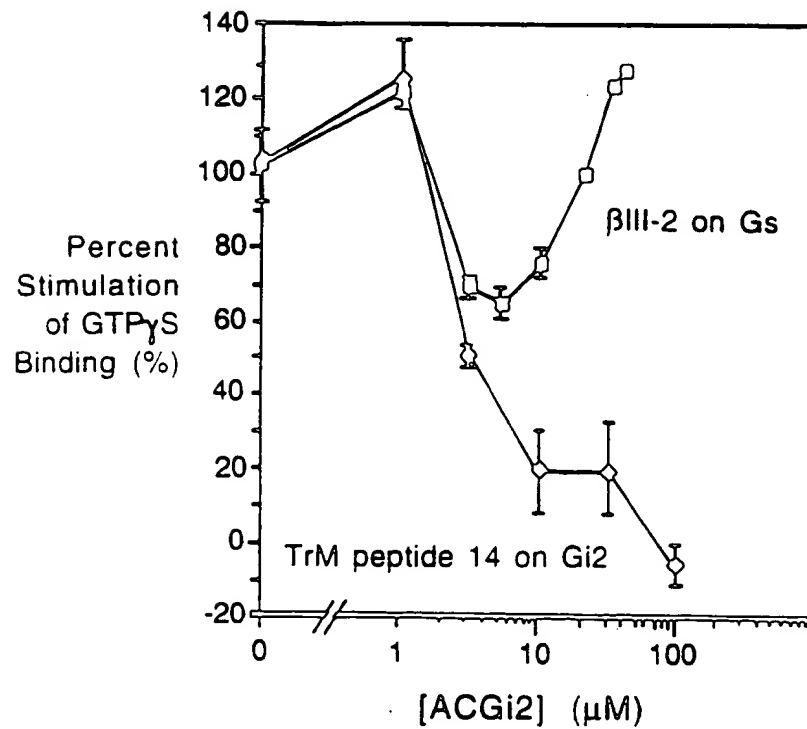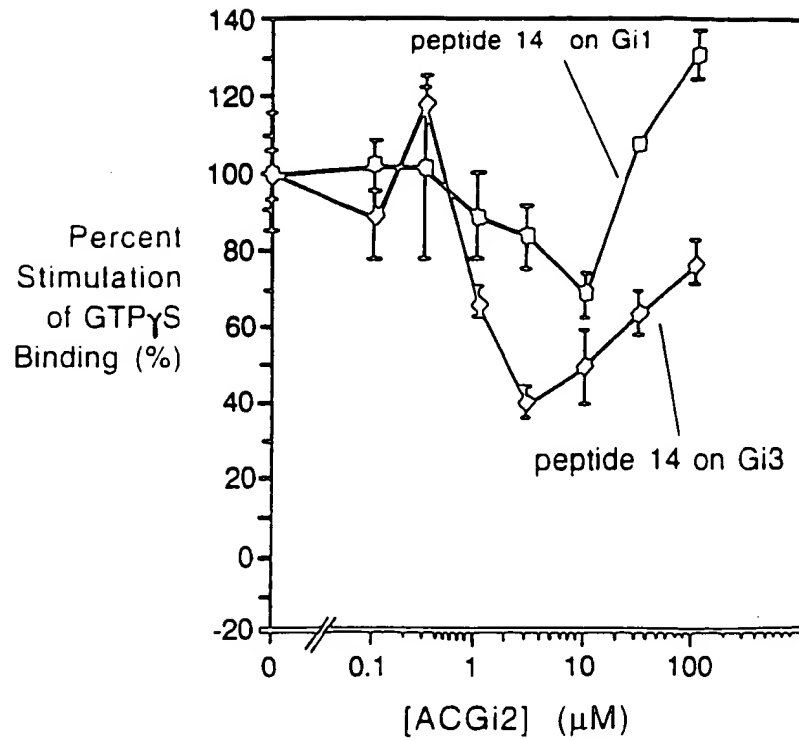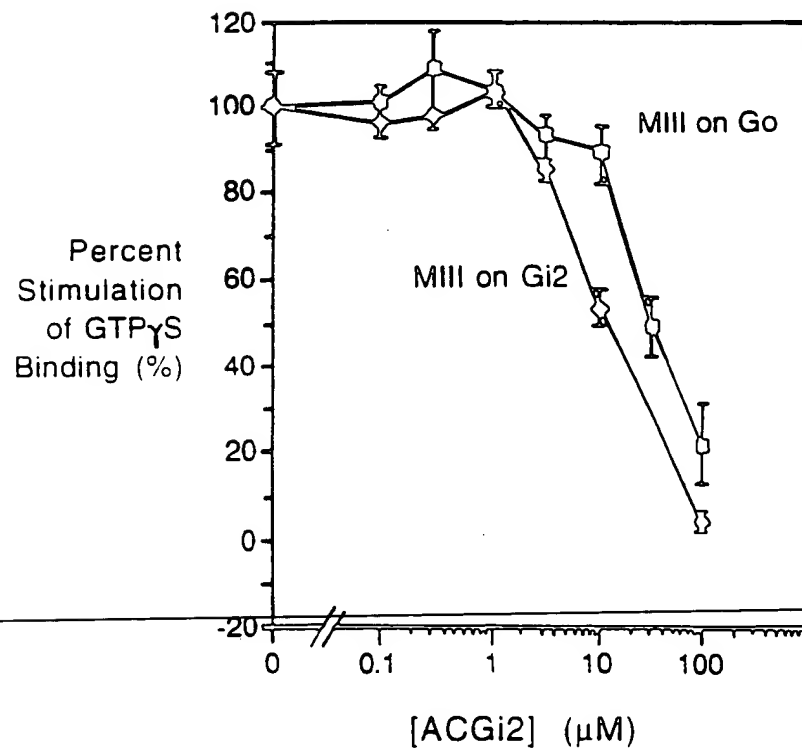
FIG. 1A

FIG. 1B

FIG. 1C

FIG. 1D
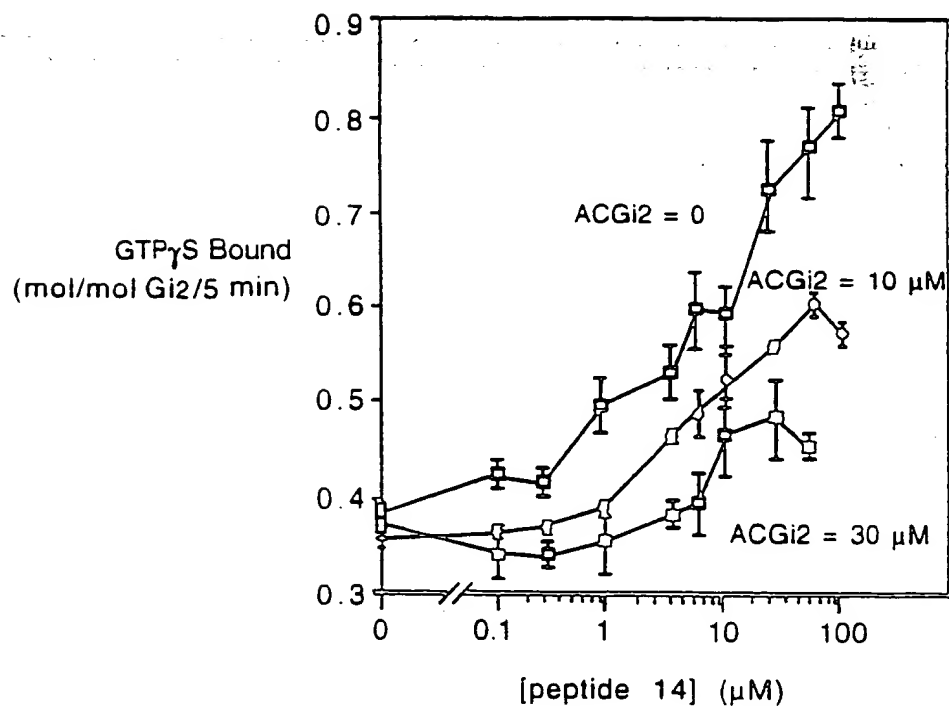
FIG. 2A
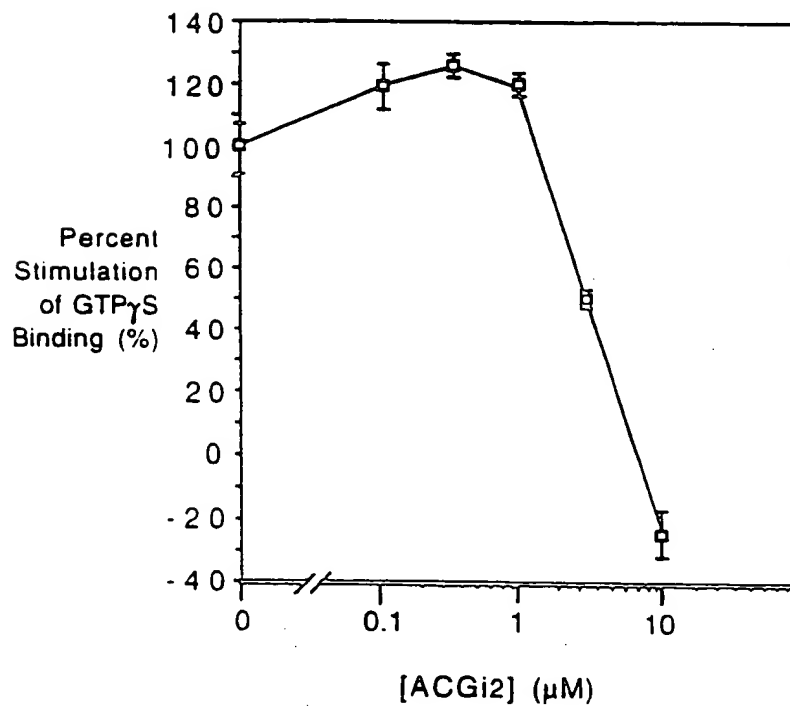
FIG. 2B
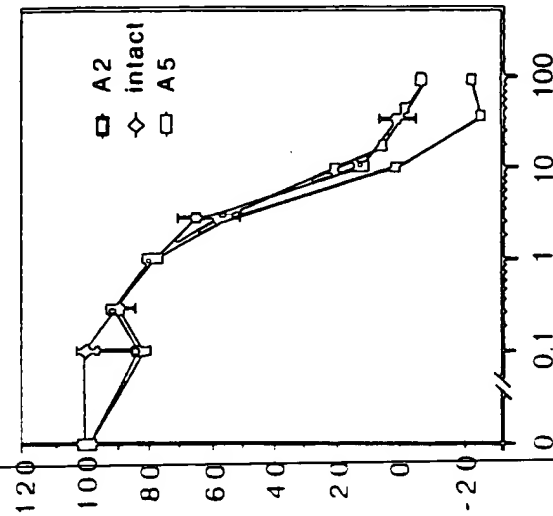
FIG. 3C

FIG. 3B

FIG. 3A
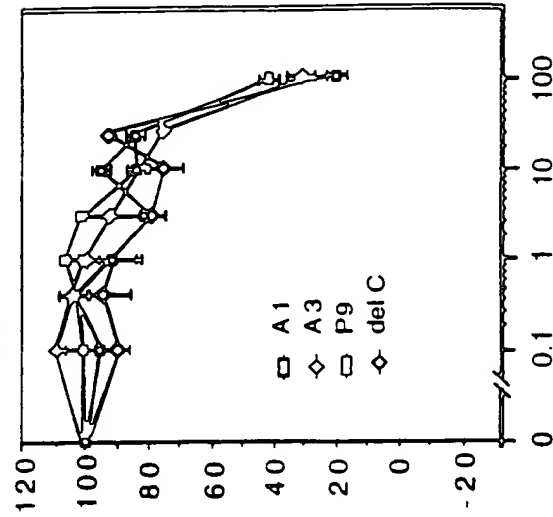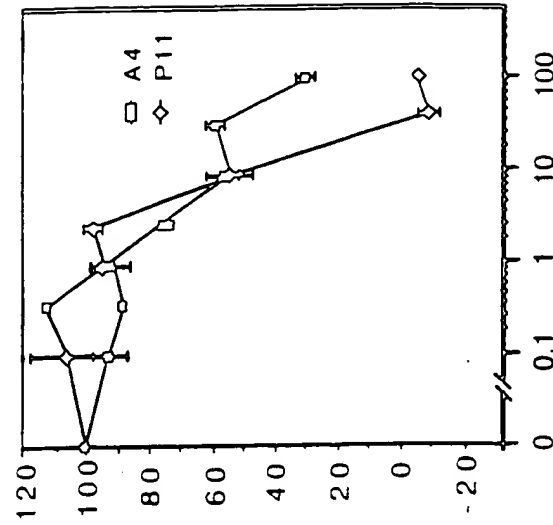
FIG. 4

Rate Constant
of
GTPγS Binding (min-1)
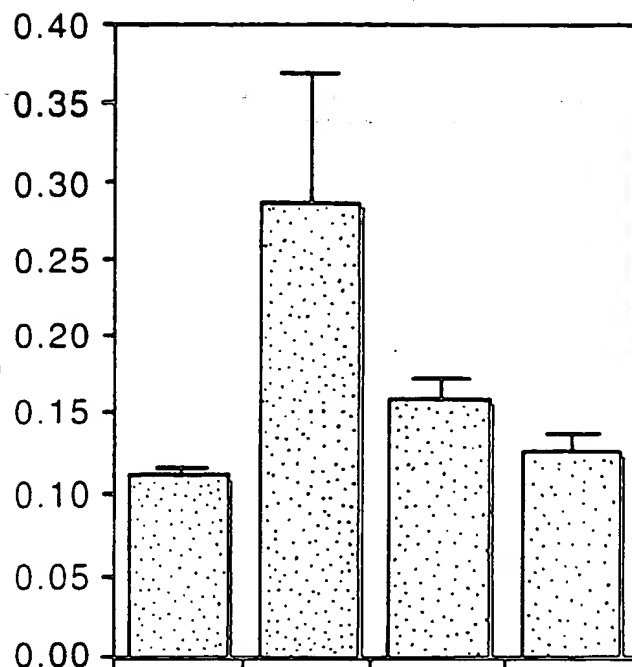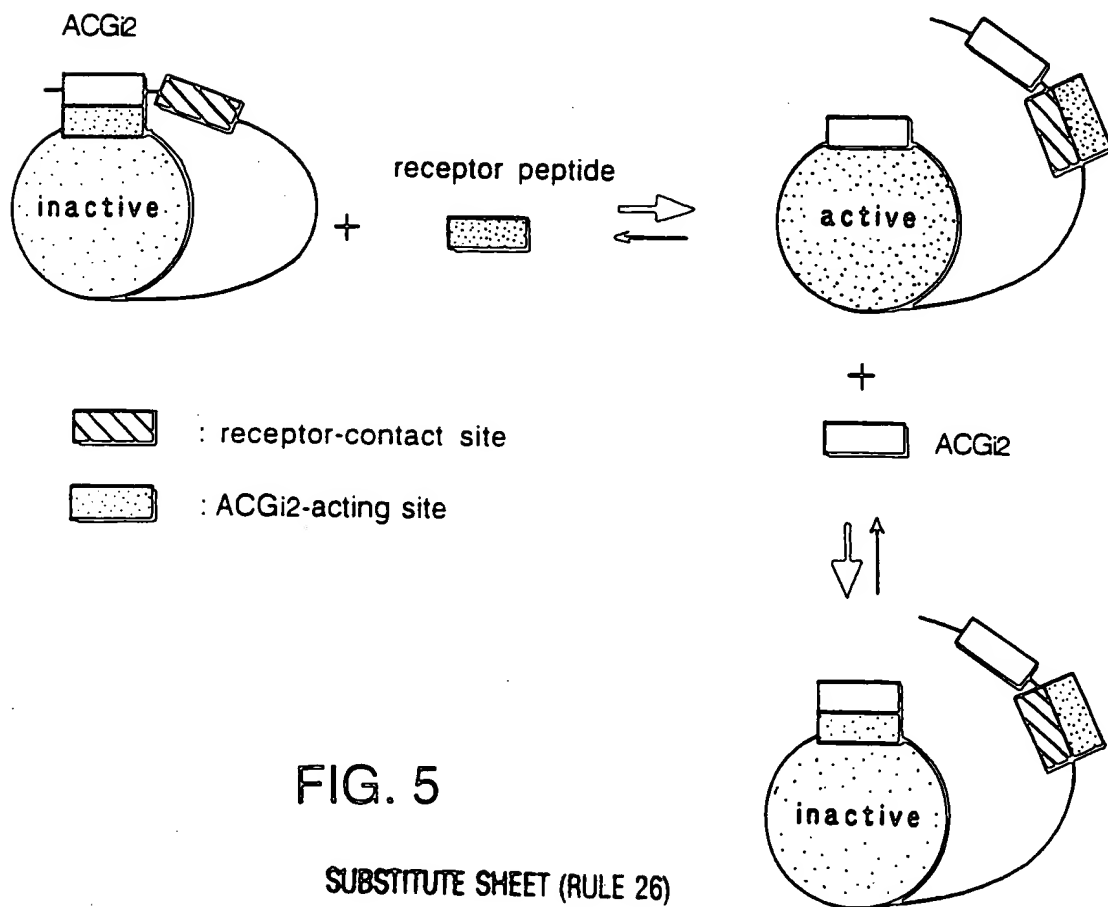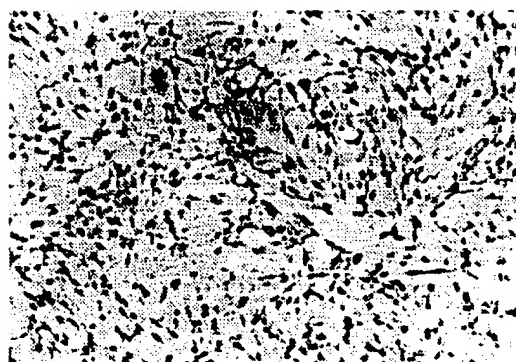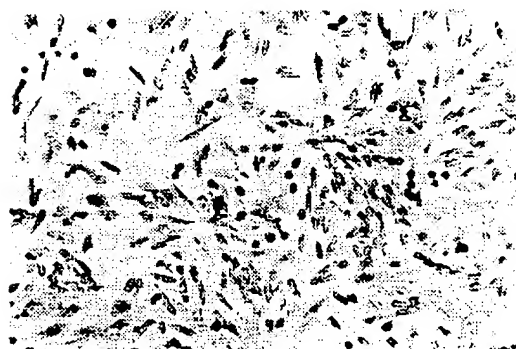

| ACh (10 mM) | − | + | + | − |
| ACGi2 (30 μM) | − | − | + | + |

FIG. 5
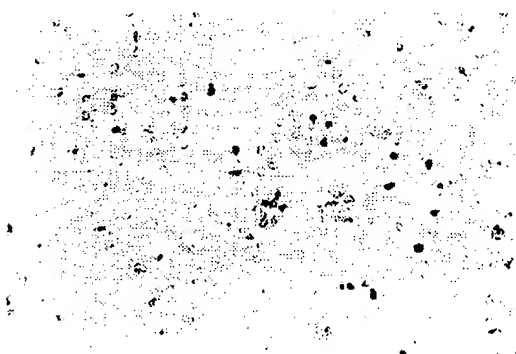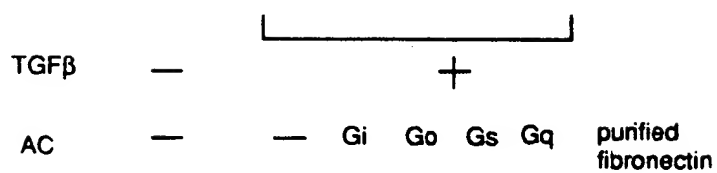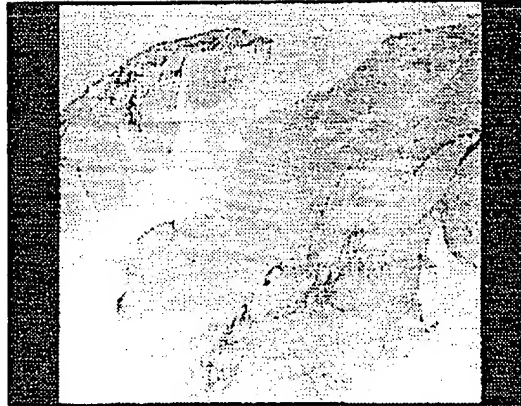
0 μM
ACGi2

10 μM
ACGi2

100 μM
ACGi2

FIG. 6

FIG. 7

Control

+ ACGi2

FIG. 8

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 37/02: CO7K 7/08, 7/10

US CL : 530/324, 325, 326

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 325, 326

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Science, Vol. 249, issued 10 August 1990, Lyons et al, "Two G Protein Oncogenes in Human Endocrine Tumors", pp. 628-631.	1-28
A	Journal of Biological Chemistry, Vol. 264, No. 24, issued 25 August 1989, Nishimoto, et al, "Possible Direct Linkage of Insulin-like Growth Factor-II Receptor with Guanine Nucleotide-Binding Proteins", pp 14029-14038.	1-28

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 MAY 1994	JUN 17 1994

Name and mailing address of the ISA/US	Authorized officer
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